ISOLATION AND CHARACTERIZATION OF SINGLE USE PLASTIC DEGRADING MICROORGANISMS FROM PETROLEUM CONTAMINATED SITE

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

IN

MICROBIOLOGY

Submitted By

Submitted by-SNEHADRITA SENGUPTA

Registration ID: 230010034742

Enrolment ID: 2322211001003

Advisor

Dr. Swati Chakraborty

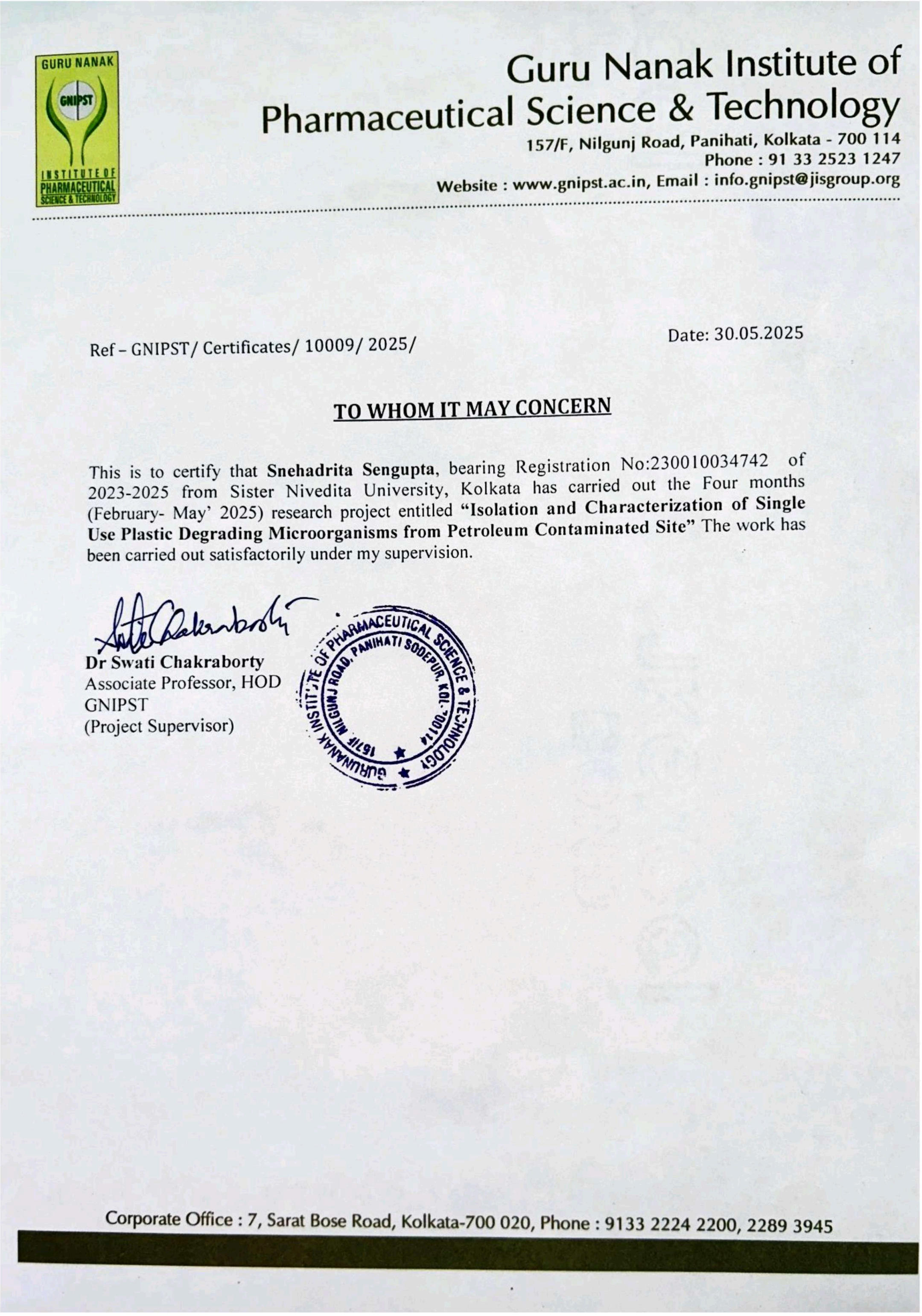
Associate Professor of Department of Life Sciences

School of Health Science and Translational Research

Sister Nivedita University

Kolkata

June 2025



Guru Nanak Institute of
Pharmaceutical Science & Technology
157/F, Nilgunj Road, Panihati, Kolkata - 700 114
Phone : 91 33 2523 1247
Website : www.gnipst.ac.in, Email : info.gnipst@jisgroup.org

Ref – GNIPST/ Certificates/ 10009/ 2025/

Date: 30.05.2025

TO WHOM IT MAY CONCERN

This is to certify that **Snehadrita Sengupta**, bearing Registration No:230010034742 of 2023-2025 from Sister Nivedita University, Kolkata has carried out the Four months (February- May' 2025) research project entitled **"Isolation and Characterization of Single Use Plastic Degrading Microorganisms from Petroleum Contaminated Site"** The work has been carried out satisfactorily under my supervision.

Dr Swati Chakraborty
Associate Professor, HOD
GNIPST
(Project Supervisor)

Corporate Office : 7, Sarat Bose Road, Kolkata-700 020, Phone : 9133 2224 2200, 2289 3945

DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Isolation and characterization of Single-use Plastic degrading microrganisms from petroleum contaminated site." in partial fulfilment of the requirements for the award of the degree of Masters in Microbiology is an authentic record of my own work carried out during the period from February,2025 to May,2025 under the supervision of Dr. Swati Chakraborty, Associate Professor, Department of Life Sciences.

I acknowledge that I have read and understood the UGC regulations, 2018 (Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions).

I confirm that this dissertation has been checked for plagiarism using the plagiarism checking software SMALL SEO TOOL. At the end of the dissertation, a copy of the summary report demonstrating similarities in content and its potential source (if any), generated online using plagiarism checking software is enclosed. I herewith confirm that the dissertation has been less than 10% similarity according to the plagiarism checking software's report and meets the UGC Regulations as well as plagiarism rules of Sister Nivedita University.

I further declare that no portion of the dissertation or its data will be published without the Institute's or supervisor's permission. I have not previously applied for any other degree or award using the topics and findings described in my dissertations.

Snehadrita Sengupta
05/06/2025

(Signature of the student with Date)

Name of the Student- SNEHADRITA SENGUPTA

Registration no.-230010034742

Department-MICROBIOLOGY

SISTER NIVEDITA UNIVERSITY
সিস্টার নিবেদিতা বিশ্ববিদ্যালয়
DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

TO WHOM IT MAY CONCERN

This is to certify that the project report titled "Isolation and Characterization of Single Use Plastic Degrading Microorganisms from Petroleum Contaminated Site", submitted by Ms. Snehadrita Sengupta (Registration No. 230010034742, Enrolment No 2322211001003) to the Department of Microbiology/Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by him/her for the partial fulfillment of the requirements for the Master of Science degree.

Date: 5/06/25
Place: Newtown, Kolkata

Signature of Head of the Department
Seal

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all those who have supported and guided me throughout the course of this thesis.

First and foremost, I am deeply indebted to my supervisor, Dr. Swati Chakraborty, Associate Professor, Guru Nanak Institute of Pharmaceutical Science and Technology, for her invaluable guidance, encouragement, and unwavering support throughout the duration of this research. Her expertise, insightful feedback, and constant motivation were instrumental in shaping the course of this work.

I would also like to extend my heartfelt thanks to Dr. Rajat Pal, Associate Professor, Sister Nivedita University, for providing us with the opportunity and resources to conduct this research.

I am equally grateful to the faculty members and staff of the Department of Life Sciences, Guru Nanak Institute of Pharmaceutical Science and Technology, for their continuous support, valuable suggestions, and access to facilities that significantly contributed to the completion of this thesis.

My sincere appreciation goes to my friends and laboratory colleagues for their camaraderie, moral support, and for sharing both the challenges and memorable moments of this academic journey.

Above all, I am profoundly thankful to my family for their unconditional love, patience, and encouragement. Their unwavering belief in my abilities provided me with the strength to persevere through every obstacle.

Finally, I extend my appreciation to all those who, directly or indirectly, contributed to the successful completion of this thesis. This work is a reflection of their collective support and contributions.

LIST OF FIGURES

FIGURE NUMBER	TITLE	PAGE NUMBER
1.	Introduction	14
2.	Instrumentation	26-28
3.	Methodology- Soil sample collection	29
4.	Results-	
	• Soil sample collection	34
	• Pure culture isolation of microbes from petroleum contaminated soil sample was done by performing serial dilution.	34
	• Pure culture isolation of Milk BRS11 provided by the lab by streak plate method.	35
	• Morphological characterization was performed by assessing the colony morphology of the isolated bacteria.	35-36
	• Morphological characterization of isolated microbes by gram staining method.	37
	• Biochemical identification of the isolated microbes-	
	➢ Catalase test	38-39
	➢ Citrate Utilization test	39-40
	➢ Gas production from glucose test	40-42

LIST OF TABLES

TABLE NUMBER	TITLE	PAGE NUMBER
1.	Morphological characterization of isolated microbes by assessing the colony morphology	35-36
2.	Morphological characterization of isolated microbes by gram staining method	37
3.	Biochemical characterization of isolated microbes –	
	➢ Catalase test	38-39
	➢ Citrate Utilization test	39-40
	➢ Gas production from glucose test	41-42

ABBREVIATIONS

NA- Nutrient Agar

PE- Polyethylene

OD- Optical Density

PET- Polyethylene Tetraphthalate

ABSTRACT

Plastic pollution, particularly from single-use plastics like polythene bags and packaging materials, has become a major global environmental concern. These materials are used widely due to their convenience, but their resistance to natural degradation makes them especially harmful to ecosystems. Once discarded, they persist in soil and water for decades, posing risks to wildlife, ecosystems, and human health.

In search of sustainable solutions, scientists are increasingly exploring biological methods to deal with plastic waste. One promising approach is using microbes to break down plastic polymers. Certain bacteria and fungi produce enzymes capable of degrading plastics into simpler, less harmful substances. This study focuses on identifying and evaluating the plastic-degrading potential of bacteria, particularly those found in challenging environments like petroleum-contaminated soil.

Soil samples were collected from a hydrocarbon-rich site like garages, which is likely to harbor bacteria that have adapted to break down complex carbon compounds like polythene. After serial dilution, a promising bacterial isolate from the 10-3 dilution, which was named as Sn1, was selected for further study. Two additional bacterial strains(Milk BRS11 and C1) were provided by the GNIPST lab. Each strain was cultured on nutrient agar plates, with small (5 mg) pieces of single-use polythene added as the only available substrate. The setup was incubated at 37 degree celcius for 30 and 45 days under controlled conditions to monitor the breakdown of single-use plastic over time.After 30 days of incubation, C1 showed significant result, indicating complete degradation of single-use plastic discs. Milk BRS11 and Sn1 shows a partial degradation of single use plastic discs. After 45 days of incubation, C1 again showed significant result, indicating complete degradation of single-use plastic

discs. Milk BRS11 and Sn1 shows further reduction weight indicating slower degradation of single-use plastic discs.The order of bacterial strains degrading single use plastic discs- C1>Sn1>Milk BRS11.

Standard biochemical tests were performed the understand effectively how the bacterial isolates function. This preliminary study suggests that certain soil bacteria, especially those from contaminated environments, may possess the natural ability to degrade single-use plastics. They are typically produced from different synthetic polymers obtained from fossil fuels. Polyethylene, polypropylene, polystyrene, phthalates etc are used in single-use plastics.

KEYWORDS

Single use plastic; plastic degrading microbes; Polyethylene tetraphthalate (PET); bioremediation; microbial screening.

CONTENTS

SERIAL NUMBER	PARTICULARS	PAGE NUMBER
1.	Introduction	
	• Plastics affect different layers of soil.	13-14
	• Types of plastics available	15
	• Microbial mechanism for plastic degradation	15-17
2.	Review of literature	18-24
3.	Aims and objectives	25
4.	Instrumentation	26-28
5.	Methodology	
	• Soil sample collection	29
	• Insulation of bacteria from petroleum defiled soil sample	29
	• Identification of insulated microbes from petroleum- contaminated soil sample and the cultures provided by the lab	29-30
	• Biochemical characterization of insulated microbes-Catalase test, Citrate utilization test, Gas production from glucose test.	30-33
	• Evaluation of the efficiency of single used plastic degradation property by selected microbial isolates under laboratory condition	33
6.	Results	34-43
7.	Discussion	44-46
8.	Conclusion	47-48
9.	References	49-50
10.	Plagiarism Report	

INTRODUCTION

Plastics have converted modern life due to their strictness, durability, and affordability. They play a vital part in everyday life, showing up in everything from food packaging and structure paraphernalia to husbandry tools and medical supplies. still, the truly parcels that make plastics so useful — particularly their resistance to declination — have turned them into one of the most patient environmental pollutants. While plastic pollution in abysms constantly grabs captions, the impact on terrestrial ecosystems, especially soil, tends to be overlooked despite being equally serious. Soil is the foundation of life on Earth, furnishing the support and nutrients that shops, brutes, and entire ecosystems depend on. It nurtures plant growth, houses different microbial communities, regulates water flux, and stores carbon. When plastics enter the soil, they can physically block water absorption and root growth, disrupt microbial communities, and leach toxic chemicals analogous as phthalates, stabilizers, and heavy substance. Over time, plastics also break down into micro- and nanoplastics, which can enter the food chain and further disturb ecological balance. These multi- faceted impacts — physical, chemical, and natural — underscore the critical need for effective and sustainable results to soil plastic pollution.

Plastics affect different layers of soil-

When plastic gets into the soil, it does not just stay on the face it can work its way down into different layers of the ground, and each subcaste feels the impact in its own way.

Top Layer (Topsoil – where plants grow)

This is where utmost most plastic ends up first—bits from plastic bags, bottles, or even tiny particles from things like agricultural films. In this subcaste:

The plastic messes with how the soil holds water and air, making it harder for factory roots to breathe and grow.

bitsy brutes like earthworms and insects, which help keep the soil healthy, might eat the plastic by mistake. This can harm them and decelerate down their work of breaking down organic matter and recycling nutrients.

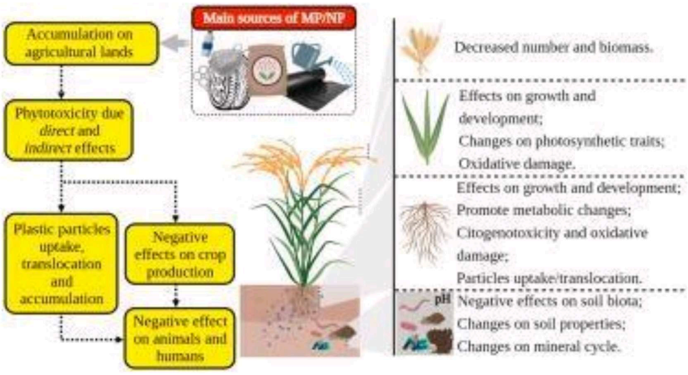

Main sources of MP/NP
Accumulation on agricultural lands
Phytotoxicity due direct and indirect effects
Plastic particles uptake, translocation and accumulation
Negative effects on crop production
Negative effect on animals and humans
Decreased number and biomass.
Effects on growth and development;
Changes on photosynthetic traits;
Oxidative damage.
Effects on growth and development;
Promote metabolic changes;
Citogenotoxicity and oxidative damage;
Particles uptake/translocation.
pH
Negative effects on soil biota;
Changes on soil properties;
Changes on mineral cycle.

Figure 1 Source- ScienceDirect.com

Middle Layer (Subsoil – just below the surface)

As time passes, rain and factory roots can push plastic deeper.

• Then, the plastic starts to mess with the balance of bacteria and fungi that

live in the soil. These microbes are pivotal for effects like putrefying waste and helping shops get nutrients.

• Chemicals from the plastic can also strain out and mix with the soil, changing its natural makeup.

Deep Soil (Below 60 cm – where roots rarely reach)

Once plastic gets this deep, it sticks around for a long time.

• This part of the soil does not have as important microbial life, so plastic breaks down indeed more sluggishly.

• It can change how water moves through the soil, making it harder for deeper layers to store water or support factory roots.

• Over time, plastic can make up then still, conceivably causing long- term damage we do not completely understand yet.

Types of plastics available-

Utmost plastic waste comes from synthetic polymers specifically designed for continuity and long shelf life. Some of the most generally used plastics are polyethylene (PE), polypropylene (PP), polystyrene (PS), PET (used in drink bottles), and PVC (frequently set up in pipes and lines). These polymers are the backbone of innumerous everyday products, from single- use shopping bags and water bottles to food wrappers, agrarian flicks, toys, and construction accoutrements.

Among these, single- use polyethylene — particularly low- viscosity polyethylene (LDPE) — is one of the most wide and environmentally patient accoutrements. Its inflexibility and strength make it useful, but also delicate to break down. In numerous corridor of the world, especially in developing regions where waste operation structure is limited, these plastics end up in open tips, water bodies, or mixed with organic waste in agrarian soils. When buried in soil, they intrude with natural processes and damage soil structure. Given the scale of the problem and the growing accumulation of synthetic waste, there's a pressing need to identify and develop effective declination strategies for these accoutrements (Hopewell, J., Dvorak, R., & Kosior, E.(2009). Plastics recovering challenges and openings. Philosophical Deals of the Royal Society B Biological lores, 364(1526), 2115 – 2126.)

Microbial Mechanisms for Plastic Degradation

Nature provides a promising path forward through the action of microorganisms. Certain bacteria and fungi have acclimated to weakened surroundings by developing mechanisms to degrade synthetic polymers. Microbes similar *as Pseudomonas, Bacillus, Aspergillus*, and *Penicillium* have demonstrated the capability to break down plastics by producing enzymes that target the chemical bonds in polymer chains.

Microbial plastic declination is a gradational and complex process that generally unfolds in four connected stages. It begins with face colonization, where microorganisms attach themselves to the plastic face and form a biofilm. This biofilm acts as a defensive and functional subcaste, allowing the microbes to establish a stable terrain for growth and enzyme stashing. Once the biofilm is in place, the microbes start producing enzymes similar as hydrolases and oxygenases that initiate the breakdown of the long- chain polymer structures of plastic in a process known as depolymerization. These enzymes stick the plastic into lower, more manageable fractions like oligomers and monomers.

Following depolymerization, the third stage assimilation — takes place. Then, the microbes absorb the lower breakdown products, using them as sources of energy and carbon to support their metabolism and growth. This is a pivotal step, as it transforms the inert plastic- deduced motes into biologically usable forms. Eventually, the process culminates in mineralization. In aerobic surroundings, the declination results in the product of carbon dioxide, water, and microbial biomass. still, under anaerobic conditions where oxygen is limited — methane may also be released as a derivate. This entire sequence reflects the remarkable capability of certain microorganisms to not only survive on synthetic accoutrements but also contribute to the breakdown and recycling of plastic waste in the terrain.

Although complete mineralization of plastics takes a long time, these microbial processes show promising eventuality for long- term declination, especially when optimized. still, several environmental factors similar as temperature, pH, humidity, and the vacuity of other nutrients — can impact the declination rate. also, we still have limited knowledge about how these microbes interact with the soil ecosystem during the declination process.

This study focuses on segregating and assaying bacteria from petroleum- defiled soil, an terrain likely to harbor organisms formerly acclimated to breaking down hydrocarbon- suchlike substances. The thing is to assess their eventuality for demeaning single- use polyethylene and to explore any associated changes in soil health. By understanding these microbial relations, we can take important way toward developingeco-friendly results for managing plastic waste in terrestrial ecosystems. (Shah, A. A., Hasan, F., Hameed, A., & Ahmed, S. (2008). Biological declination of plastics A comprehensive review.Biotechnology Advances, 26(3), 246 – 265.)

REVIEW OF LITERATURE

1. **Saritha et al. (2021) -** ***Plastic Degrading Microbes: A Review*** **Microbiology Research Journal International**

   Saritha and associates (2021) handed a foundational review of microbial mechanisms involved in the declination of synthetic plastics. Their work laid out the growing concern of plastic accumulation in ecosystems and the eventuality for natural agents, particularly microbes, to alleviate this issue. The review distributed plastic types by degradability and explored both bacterial and fungal agents known to retain plastic- demeaning capabilities. For case, Pseudomonas, Streptomyces, and Bacillus were stressed for their places in polyethylene declination, while fungi similar as Aspergillus niger and Penicillium spp. showed pledge in breaking down polyurethane. The authors emphasized the part of specific enzymes, similar as laccases and cutinases, in depolymerizing plastic motes and transubstantiating them into lower dangerous composites. Their work handed a strong theoretical background for the microbial bioremediation of plastic waste and established directions for unborn applied exploration.

2. **Srikanth et al. (2022)** ***–Biodegradation of plastic polymers by fungi: a brief review*****, Bioresources and Bioprocessing**

   Srikanth, Sandeep, and Sucharitha(2022) concentrated on the practical insulation and characterization of fungi able of demeaning plastic flicks. Working with soil samples collected from plastic- defiled spots, the platoon successfully insulated strains of Aspergillus and Fusarium that demonstrated the capability to break down polyethylene over a period of 30 days. The extent of plastic declination was assessed by measuring weight loss and examining chemical changes using FTIR spectroscopy, along with imaging face damage through surveying electron microscopy(SEM). Clear substantiation of physical and chemical deterioration of plastic flicks was proved, including face corrosion and changes in functional groups. This study showcased not only the presence of plastic- demeaning fungi in terrestrial surroundings but also their eventuality for development intobio-based plastic waste treatment technologies. The authors concluded that fungal biodegraders are a promising and underexplored resource for sustainable waste operation.

3. **Ali et al. (2023) -*Characterization of plastic degrading bacteria isolated from sewage wastewater*, Saudi Journal of Biological Sciences**

   Ali and his platoon(2023) explored sewage wastewater as a force for plastic-demeaning microbes, particularly fastening on Pseudomonas spp. The study insulated two strains — SH5B and SH6B — from wastewater and tested them on polyethylene substrates. These strains displayed clear zone conformation on plastic- supplemented agar plates, signifying declination exertion. farther analyses, including FTIR and weight loss measures, verified that the plastics passed substantial structural changes due to microbial exertion. This exploration illustrated how civic and artificial waste surroundings can harbor potent biodegrading bacteria. The authors emphasized that similar naturally being strains, if farther characterized genomically and enzymatically, could be employed ineco-friendly waste treatment systems. Their work corroborated the idea that pollution hotspots might serve as rich sources of microbial biodiversity with operations in environmental biotechnology.

4. **Purohit et al. (2020) - *Metagenomic Exploration of Plastic Degrading Microbes for Biotechnological Application*, Current Genomics**

   Purohit, Chattopadhyay, and Teli(2020) espoused a genomic perspective to study microbial plastic declination. Using metagenomics and bioinformatics, the experimenters anatomized tip microbial communities and linked colorful genes rendering for enzymes like hydrolases, oxidases, and esterases each involved in plastic declination. Their genome sequencing sweats revealed numerous unculturable microbes that may play a central part in demeaning polyethylene and PET(polyethylene terephthalate). The study stressed how advanced' omics' tools, like metagenomics, can go beyond the limits of traditional lab societies, helping us discover new microbes and genes involved in plastic declination. This approach allows scientists to identify preliminarily unknown declination pathways and microbial agents, furnishing tools to mastermind more effective bioremediation systems.

5. **Bandopadhyay et al.(2020) –** ***Soil Microbial Communities Associated With Biodegradable Plastic Mulch flicks*****, borders in Microbiology**

   Bandopadhyay and associates(2020) delved how biodegradable plastic mulch flicks impact soil microbial communities. Through field trials, they observed changes in microbial biomass, enzyme exertion, and species diversity in soils treated with biodegradable mulches compared to traditional plastics. Interestingly, while these biodegradable accoutrements did n't persist in the soil, they still told microbial race and functional diversity. The study refocused out that indeed Eco-friendly plastic druthers can alter soil ecosystems in subtle but important ways. Their exploration stresses the significance of long- term monitoring and comprehensive ecological assessments before espousing new biodegradable accoutrements at scale.

6. **Shah et al.(2008*****) Biological declination of plastics A comprehensive review*****, Biotechnology Advances**

   This paper takes a close look at how microbes break down different types of synthetic plastics like polyethylene, polypropylene, polystyrene, and PVC. The authors explain that enzymes similar as hydrolases, lipases, and esterases play a crucial part in cutting the long plastic chains into lower pieces. They also point out that effects like how tightly packed the plastic motes are(called crystallinity), the size of the plastic motes, and environmental factors all impact how presto the plastics break down. Interestingly, they emphasize that groups of microbes working together(microbial colleges) tend to be more effective at demeaning plastics than single strains working alone.

7. **Yoshida et.al.(2016)-** ***A bacterium that degrades and assimilates poly(ethylene tetraphalate),*** **Science**

   Yoshida and his platoon discovered a remarkable bacterium called Ideonella sakaiensis that can actually “ eat ” PET plastic and use it as its only source of food. They set up that this microbe produces two special enzymes, PETase and MHETase, which break down the plastic into lower structure blocks that the bacteria can also digest. This was a huge advance because it revealed a natural way to attack a major plastic contaminant and opened up instigative possibilities for using these microbes in managing plastic waste more effectively.

8. **Pathak and Navneet(2017*)- Review on the current status of plastic biodegradation and its impact on terrain*, Environmental wisdom and pollution exploration**

   This paper looks at the rearmost progress in how bacteria and fungi from plastic- defiled surroundings can break down plastics. It also talks about how plastics affect the terrain, especially how they pollute soil and produce bitsy plastic patches called microplastics that can mess up the natural balance of microbes and nutrients in the soil. The authors suggest that if we manage microbial plastic declination well, it could n't only help reduce plastic pollution but also help restore the health of the soil.

9. **Arkatkar et.al.(2010)-** ***Insulation of plastic demeaning bacteria and their impact on soil parcels*****, Journal of Environmental wisdom and health**

This study set up bacteria from weakened soils that can break down plastics, and also looked at how this process affected the soil. They noticed that after the plastics were broken down, important soil enzymes came more active and nutrient situations bettered. This suggests that microbes breaking down plastics can actually help ameliorate the soil’s health and overall quality.

10. **Dwivedi and Mishra(2022)-*Plastic demeaning microbes A review on their operations and impact on soil ecosystem,* Environmental wisdom and pollution exploration**

    The authors look at different microbes and groups of microbes that are good at breaking down plastics, and they explore how this affects the variety and conditioning of microbes living in the soil. They suggest that when microbes break down plastics, they can help bring back important soil enzymes and nutrient cycles that plastic pollution had disturbed, which in turn supports the overall health of the soil ecosystem.

11. **Shimao(2001)- *Biodegradation of plastics*, Current opinion in biotechnology**

    This early review laid the foundation for understanding how microbes break down plastics. It explains how microbes interact with synthetic plastics, the enzymes they use to do this, and the environmental factors that impact the process. This work helped pave the way for exploring how microbes could be used to manage plastic waste more effectively.

12. **Ojha et.al.(2021)- *Biodegradation of plastics by fungi and bacteria A sustainable approach,* Microbial biotechnology**

    This paper highlights how fungi like Aspergillus and Penicillium, along with bacteria, play an important part in breaking down plastics. It explains how fungal enzymes similar as laccases and peroxidases help in this process. The authors also bandy how these microbes could be used in bioremediation sweats to clean up plastic pollution and ameliorate soil health.

13. **Rana et. al.(2019)-** ***Microbial declination of plastics and their impact on soil quality,*** **Journal of applied microbiology**

    Rana and associates looked at how microbes that break down plastics can change the makeup of soil microbial communities and affect soil nutrients. They set up that after the plastics were degraded, there was an increase in the number of microbes and in the exertion of important soil enzymes like dehydrogenase and phosphatase. This suggests that these microbes not only help reduce plastic pollution but also boost soil fertility, playing a precious binary part.

14. **Singh and Sharma (2008)-** ***Mechanistic implication of plastic degradation,*** **Journqal of polymers and the environment**

    This research dives into how microbes break down plastics at a biochemical level. It explains how enzymes like oxygenases and esterases work to break the long plastic chains into smaller parts, and how these breakdown products affect the microbes themselves. The study also points out some challenges, like how harmful substances can build up in the soil during the degradation process.

15. **Raghul et.al (2016)-** ***Isolation and characterization of plastic degrading microbes from municipal landfill,*** **International journal of environmental science and technology**

    The authors isolated bacteria from landfill soils that can break down polythene. They tested these bacteria by growing them on plastic films and confirmed the degradation by looking at changes on the plastic surface under a microscope. The study also showed that breaking down the plastic helped improve soil enzyme activities and other important soil health indicators after the microbes did their work.

16. **Tokiwa et.al. (2009)- *Biodegrdability of plastics*, International journal of molecular sciences**

    This study sorts different types of plastics based on how easily they can be broken down by microbes. It talks about the enzymes that help in this process and covers both natural plastics and synthetic biodegradable ones. The paper also explains how factors like moisture and temperature affect how well plastics break down, and it looks at how this breakdown impacts the variety of microbes in the soil and the activity of important soil enzymes.

17. **Sivan (2011)- *New perspectives in plastic buodegradation*, Current opinion in biotechnology**

    Sivan's paper explores how enzymes break down plastics like polyethylene and polylactic acid (PLA). It highlights how groups of microbes working together, along with multiple enzymes acting in synergy, can speed up the degradation process. The study also suggests that in the future, genetic engineering could be used to improve these microbes and enzymes to make plastic breakdown even more efficient.

18. **Kumar et al. (2020)- *Screening and characterization of plastic degrading bacteria from landfill sites*, Journal of environmental management**

    This study isolated several types of bacteria from landfill soils—like *Bacillus, Pseudomonas*, and *Rhodococcus*—that can break down polyethylene. The researchers confirmed the breakdown by measuring weight loss and using techniques like electron microscopy and spectroscopy to see changes in the plastic. They also emphasized how important it is to find local microbes that are naturally suited to the environment for better plastic degradation.

AIMS AND OBJECTIVES

- To insulate bacteria from petroleum defiled soil samples.
- To screen the insulated microorganisms for their implicit to degrade plastics effectively.
- To study about the morphological and biochemical characterization of insulated microorganisms
- To estimate the effectiveness of single use plastic declination property by named microbial isolates under laboratory conditions.

INSTRUMENTATION

Different instruments were used for this project work. A list of some instruments used are mentioned bellow-

1. **INCUBATOR**-

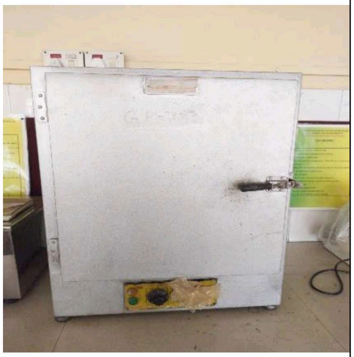


Figure 2 Incubator

Manufactured by- INDIAN INSTRUMENTS MANUFACTORING COMPANY(KOLKATA,INDIA)

Specification- Controlled growth of microorganisms

Working principle- An incubator works by keeping the environment just right for growing cells or microbes. Optimal temperature required for microbes is 37 degree Celsius. It controls the temperature, humidity, and sometimes gases like oxygen or carbon dioxide. It also makes sure the air moves evenly inside and stays clean, so the samples can grow safely and properly.

2. **LAMINAR AIRFLOW-**

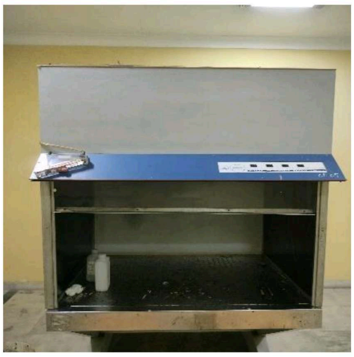


Figure 3 LaminarAirflow

Specification- Uses HEPA filters to remove particulates from the air, providing a contaminant-free environment

Working Principle- A laminar airflow system works by gently blowing super clean air in one smooth direction over your workspace. This steady airflow pushes away dust and germs, keeping the area really clean and safe. The air is cleaned through a special filter that catches tiny particles, so only pure air reaches what you’re working on.

3. AUTOCLAVE-

Specifications-

- Sterilization temperature- 121 degree Celsius
- Pressure range- 15 psi

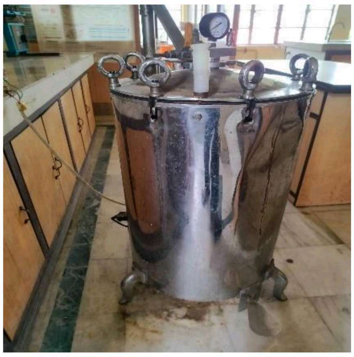


Figure 4 Autoclave

Working principle- An autoclave works like a super-hot pressure cooker. It uses steam under high pressure to heat things really well, killing all germs and bacteria. This makes sure that any tools or materials inside are completely clean and safe to use.

4. DIGITAL WEIGHING BALANCE-

Manufactured by- Shimadzu Analytical (india) private limited , Kolkata, india.

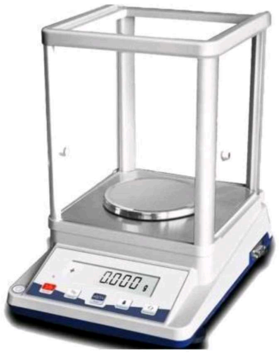


Figure 5 Digital weighing balance

Working Principle- A digital weighing balance works by feeling how heavy something is when we put it on. Inside, there's a special sensor that senses the weight and turns it into an electric signal. Then, the balance shows the exact weight as a number on the screen.

5. SPECTROPHOTOMETER-

Manufactured by- Jasco

Model number- V-630

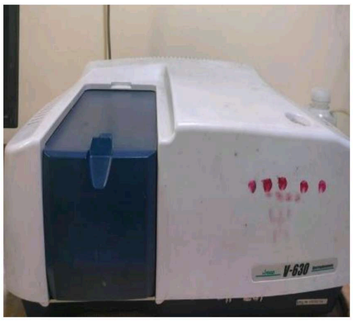


Figure 6 Spectrophotometer

Working principle- A spectrophotometer works by shining light through a sample and seeing how much light the sample soaks up. Different stuff absorbs different amounts of light, so by measuring this, the machine can tell what's in the sample or how much of it there is.

6. MICROSCOPE-

Working principle- A microscope helps us to see tiny things by making them look bigger. It uses special lenses to zoom in on objects that are too small for our eyes to see clearly. Light goes through or bounces off the object, and the lenses make a bigger, clearer picture for us to look at.

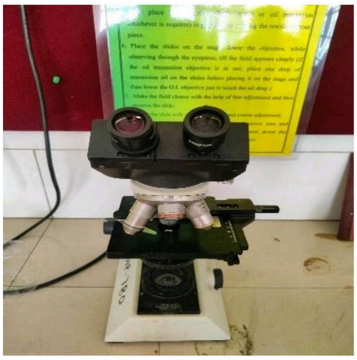


Figure 7 Microscope

METHODOLOGY

1. **Soil sample Collection-**
    - Petroleum defiled soil sample was collected from a garage for insulation of microbes
    - Two societies of bacteria- Milk BRS11 and C1 were handed by Gnipst lab

Figure 8 Petroleum defiled soil sample

2. **Insulation of bacteria from petroleum defiled soil sample-**
    - **Pure culture insulation by serial dilution**

      After the sample was collected, 1gm of it was weighed. It was serially diluted from 10- 1 to 10- 5. also 100microlitres of 10- 1, 10- 3 and 10- 5 dilutions were pipetted onto set nutrient agar plates. also it was spreaded using a glass spreader. The plates were incubated for 24 hours at 37 degree celcius.

    - **Pure culture insulation by Streak plate method**

A loopful of Milk BRS11 culture which was handed by the lab was taken and streaked on a set nutrient agar plated using a sterile inoculation loop.

3. **Identification of insulated microbes from petroleum- contaminated soil sample and the cultures provided by the lab-**

The insulated microbes were linked grounded on their appearance, growth patterns, and biochemical geste , using guidelines from Bergey’s Manual of Methodical Bacteriology(Kandler and Weiss, 1986). Each insulate was also examined through Gram staining and a series of specific biochemical tests(Albertsson, A.C. and Karlsson, S.(1990) The influence of biotic and abiotic surroundings on the declination of polyethylene. Prog Polym Sci, 15 177 – 192).

- **Morphological characterization of insulated microbes by assessing the colony morphology –**

This was done to determine the morphology of named strains on the base of shape, size and colour.

- **Morphological characterization of insulated microbes by gram staining method-**

Three clean grease free slides were taken and smears of three bacterial societies Milk BRS11, C1 and Sn1 were made on it with a sterile inoculation loop. The smears were air dried and also heat- fixed. also they were subordinated to the following staining reagents- The smears were swamped with Crystal Violet for 1 minute, followed by washing with running distilled water. Again, the smears were swamped with Gram's Iodine for 1 second which act as a mordent, followed by washing with running distilled water. also the smears were swamped with Ethyl alcohol for 30 seconds which act as a decolourizer. After that smears were stained with Safranin for 30 seconds, followed by washing with running distilled water. The slides were also air dried and cell morphology was checked under microscope.(Charu Deepika, R.Vignesh et.al. Webbing of plastic demeaning microbes from colorful ditched soil samles, International Research Journal of Engineering and Technology).

4. Biochemical characterization of insulated microbes-

Biochemical tests are simple lab trials used to figure out how the bacteria functions.. Every type of bacteria behaves else when given certain nutrients or chemicals and these responses help scientists identify them. These tests are performed to identify the bacteria, understand how they live, find out if they're dangerous or useful.

The following biochemical tests were done-

A). The catalase test was used to check the presence of catalase enzyme in all the three bacterial isolates-

Catalase test is a biochemical test used to detect the presence of catalase enzyme in a particular bacterial species Catalase decompose H2O2 into oxygen and water. This test was performed by preparing 3% hydrogen peroxide solution. This 3% H202 result was poured in three test tubes. With the help of an inoculation circle three bacterial societies- Milk BRS11, C1 and Sn1 were invested into three test tubes independently. No bubbles were formed which indicated these three societies were catalase negative as they do not produce the enzyme catalase.

B). The citrate application test was used to check if the bacterial isolates were suitable to grow by using citrate as their only source of carbon-

This test determines the capability of bacteria to convert citrate into oxaloacetate. In the citrate test, citrate is the only carbon source handed for the bacteria to use.However, they simply will not grow in the medium, If the bacteria cannot use citrate. But if they can use it, they’ll grow and start producing substances that make the terrain more alkaline. As a result, the color of the media changes to bright blue, which indicates a positive result.

To perform these test we prepared three Simmon’s citrate agar slants of the following composition-

- Ammonium dihydrogen phosphate- 0.03 g
- Dipotassium phosphate- 0.03 g
- Sodium chloride- 0.15 g
- Sodium citrate- 0.06 g
- Magnesium sulfate- 0.006 g

- Bromothymol blue- 0.0024 g
- Agar- 0.45 g
- Distilled water- 30 ml

These factors were dissolved in 30 ml distilled water and autoclaved. The media was allowed to solidify to form agar slants. These agar slants were invested with three bacterial societies- Milk BRS11, C1 and Sn1 independently with the help of sterile inoculation loop. The three slants were also incubated at 37degree celcius for 48hours. The slant societies were also observed for growth and colouration of the media.

C) The eventuality of the bacterial isolates to produce gas as a result of glucose turmoil was examined by performing gas product from glucose test-

This test was performed by preparing 100 ml of MacConkey broth. The composition of MacConkey broth-

- Peptone- 1.7 g
- Lactose- 0.3 g
- corrosiveness mariners 0.15 g
- Sodium chloride- 0.5 g
- Phenol red- 3.3 mg
- Distilled water- 100 ml

These factors were dissolved in 100 ml of distilled water in three test tubes.The durham tubes were placed inside the media in reversed condition and autoclaved. The three bacterial societies were invested into the test tubes by a sterile inoculation loop. The test tubes were incubated at 37 degree celcius for

24hours. The upward movement of reversed Durham tube indicates positive response(gas production).

5. Evaluation of the efficiency of single used plastic degradation property by selected microbial isolates under laboratory condition –

Determination of weight loss of single-use plastic by isolated bacteria-

Small plastic discs, each about 5cm in diameter and cut from single-use polythene bags, were carefully placed into conical flasks containing 50 ml of nutrient-rich culture broth. Each flask was inoculated with a different type of bacteria-Milk BRS11,C1 and Sn1 respectively, while a separate control flask—containing plastic discs but no bacteria—was set up for comparison. All the flasks were kept on a shaker to allow continuous mixing and better interaction between the microbes and the plastic. After 30days, the plastic discs were removed, thoroughly washed with distilled water, dried using filter paper, and then weighed again. Again after 45 days, the plastic discs were removed, thoroughly washed with distilled water, dried using filer paper and then weighed again. The difference in weight before and after incubation was used to calculate how much plastic had been degraded. (Charu Deepika, R.Vignesh et.al. Screening of plastic degrading microbes from various dumped soil samples, International Research Journal of Engineering and Technology).

RESULTS

1) Soil sample collection-

Figure 9 Petroleum defiled soil sample

Petroleum-contaminated soil sample was collected to isolate microrganisms to check their plastic degrading capacity.

2). Isolation of bacteria from petroleum contaminated soil sample-

- **Pure culture isolation of microbes from petroleum contaminated soil sample was done by performing serial dilution.**

After the petroleum contaminated soil sample was collected from a garage, 1gm of it was weighed. It was serially diluted from 10-1 to 10-5. 100 microlitres of 10-1, 10-3 and 10-5 dilutions were pipetted onto the nutrient agar plates. It was spreaded using a glass spreader. The plates were incubated at 37 degree celcius for 24 hours. Then the result was observed.

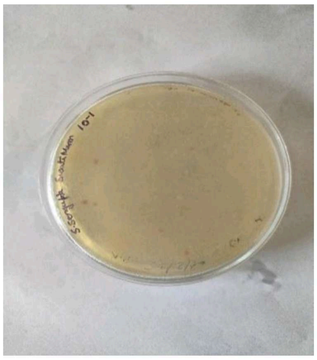


Figure 10 Serial dilution plate of 10-1

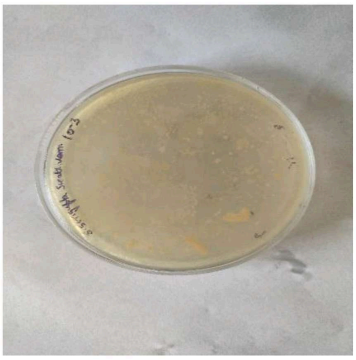


Figure 10 Serial dilution plate of 10-3

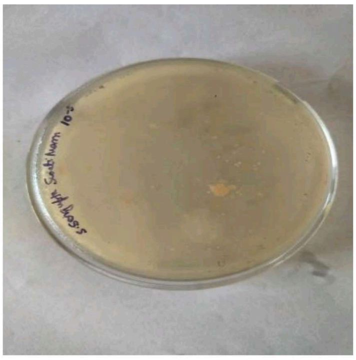


Figure 12 Serial Dilution plate of 10-5

• Pure culture isolation of Milk BRS11 provided by the lab by streak plate method.

A loopful of Milk BRS11 culture which was handed by the lab was taken and streaked onto a nutrient agar plate using a sterile inoculation loop. The plate was incubated at 37 degree elciu for 24 hours.

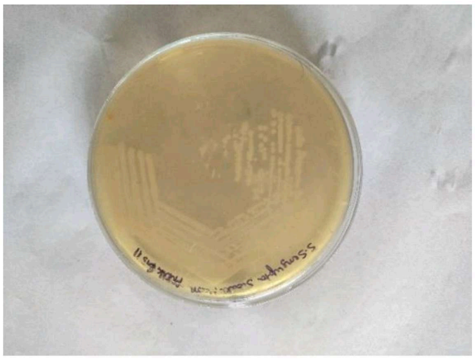
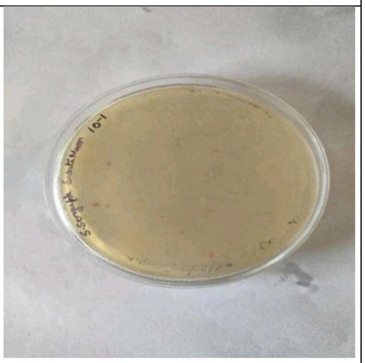
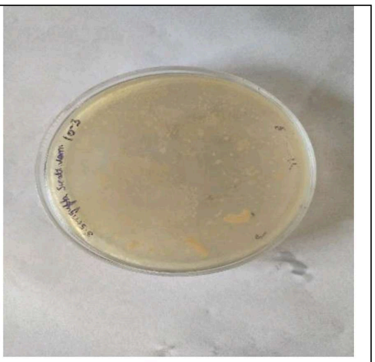
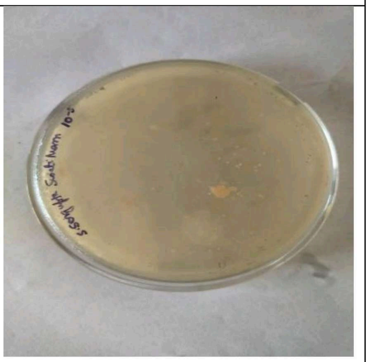


Figure 11 Streak plate of Milk BRS11

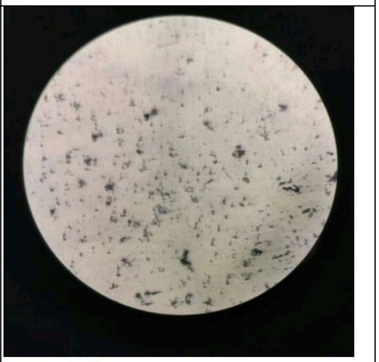
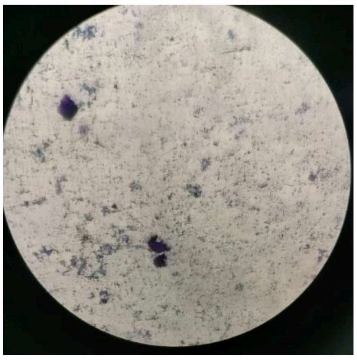
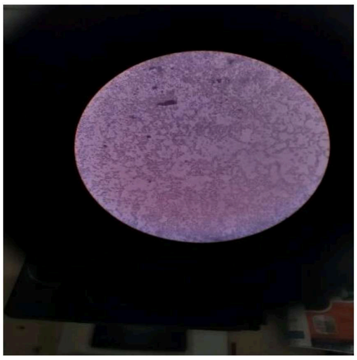
3). Identification of isolated microbes from petroleum contaminated soil sample and the cultures provided by the lab-

- Morphological characterization was performed by assessing the colony morphology of the isolated bacteria to determine the morphology of the isolated bacteria obtained by serial dilution. This was performed to determine the morphology of isolated strains on the base of shape, size and colour.

Dilution no.	Colony morphology	Source	Figure
10-1	Large round white Small round yellow Large irregular white	Petroleum contaminated soil sample.	 Figure 12 Serial dilution plate of 10-1

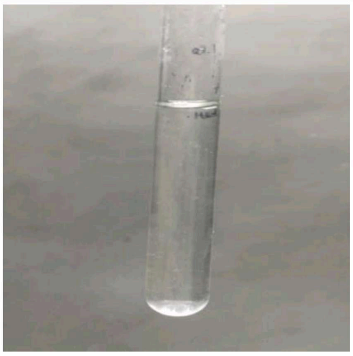
10-3	Small round white Small irregular yellow	Petroleum contaminated soil sample	 *Figure 13 Serial dilution plate of 10-3*
10-5	Small round white Small irregular yellow	Petroleum contaminated soil sample	 *Figure 14 Serial dilution plate of 10-5*

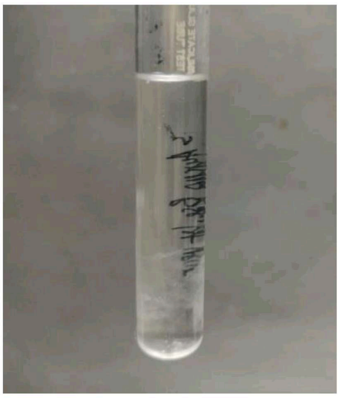
- Morphological characterization of isolated microbes by gram staining method- Gram staining was performed on Sn1which was isolated by serial dilution technique and the other two cultures provided by the lab- Milk brs11 and C1.

Bacterial strain	Shape of the organism	Colour	Characteristic	Figure
Milk BRS11	Coccus in chain	Purple	Gram positive, coccus	 *Figure 15 View of Milk BRS11*
C1	Rods in chain	Purple	Gram positive, bacillus	 *Figure 16 View of C1*
Sn1	Coccus in chain	purple	Gram positive, coccus.	 *Figure 17 View of Sn1*

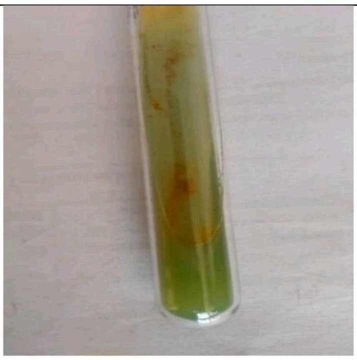
4). Biochemical characterization of isolated microbes was done. Catalase test, Citrate utilization test and gas production test were done.

A). The catalase test,was performed on all three isolates- Milk BRS11, C1 and Sn1. This test was pedrformed by preparing 3% H_2O_2 solution.This was poured in three test tubes. With the help of a sterile inoculation loop, three bacterial isolates was transferred. All the three isolates-Sn1, Milk BRS11 and C1 showed negative result.

Serial number	Catalase test	Figure
Milk BRS11	Negative	 *Figure 18 Milk BRS11 showing negative result*
C1	Negative	 *Figure 19C1 showing negative result*

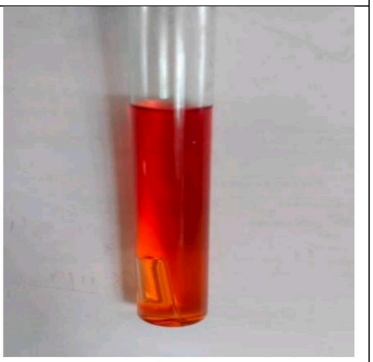
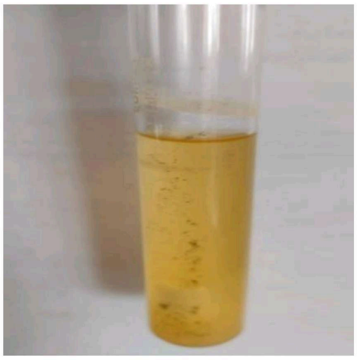
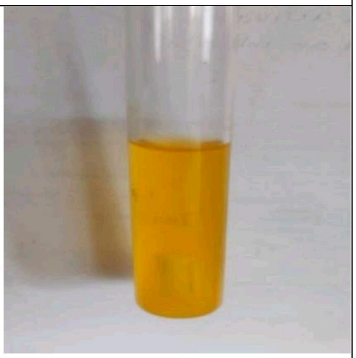
Sn1	Negative	 *Figure 20 Sn1 showing negative result*

B). For Citrate utilization test, Milk brs11 and Sn1 isolate showed negative result with no colour change of the media and c1 isolate showed positive result with colour change of the media to bright blue.

Serial number	Citrate utilization test	Figure
Milk BRS 11	negative	 *Figure 21 Milk BRS11 showing negative result*

C1	positive	 *Figure 22 C1 showing positive result*
Sn1	negative	 *Figure 23 Sn1 showing negative result*

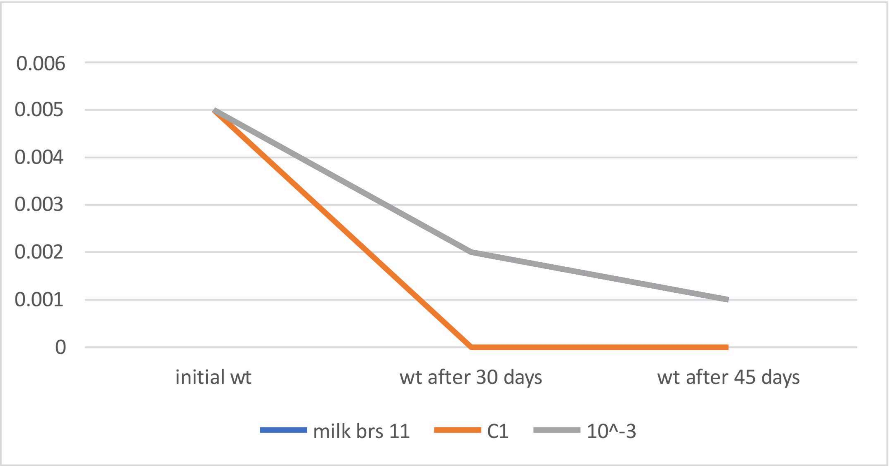
C).The gas production from glucose test was performed on all the three isolates. 100ml of MacConkey broth was prepared in which durham tubes were poured in inverted condition along with the bacterial cultures. The setup was incubated for 24 hours at 37 degree celcius. A control was taken with no bacterial culture in it and it showed negative result indicating no gas production. C1 isolate showed negative result. Milk BRS11 and Sn1 isolate showed positive result indicating gas production.

Serial number	Gas production from glucose	Figure
Control	negative	 *Figure 24 Control showing negative result*
Milk BRS 11	positive	 *Figure 25 Milk BRS11 showing positive result*
C1	Negative	 *Figure 26 C1 showing negative result*

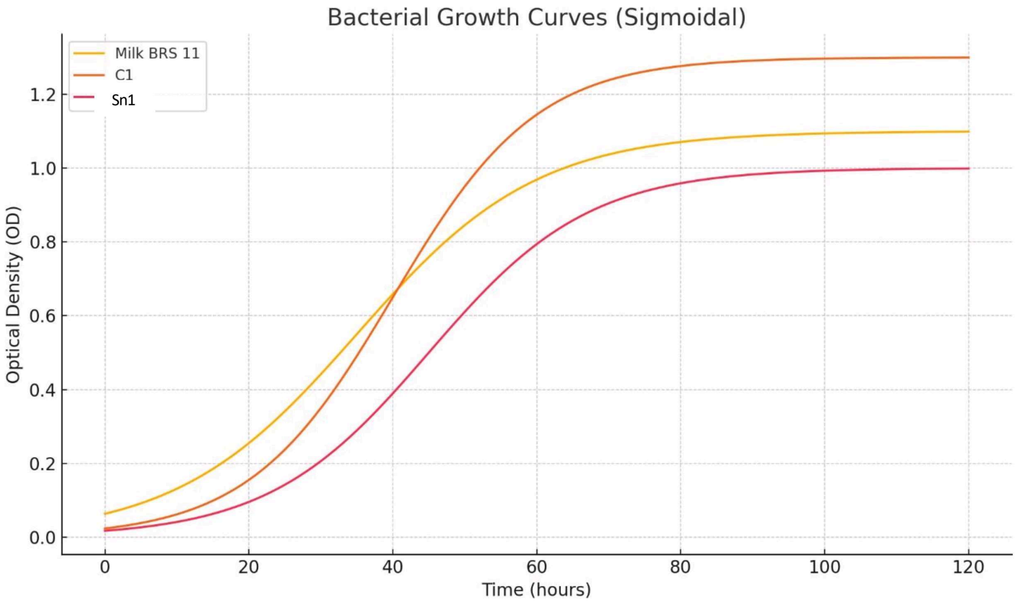
Sn1	positive	 Figure 27 Sn1 showing postive result

5). Evaluation of the efficiency of single use plastic degradation property by selected microbial isolates under laboratory conditions-

Small plastic discs of 5cm diameter were placed into conical flasks containing bacterial isolates-Sn1, Milk BRS11 and C1 and incubated at 37 degree celcius for 30days and 45 days. After 30 days C1 showed significant result, showing complete degradation of single use plastic discs. Milk BRS11 and Sn1 shows partial reduction in weight. After 45 days, C1 again showed significant result, indicating complete degrazdation of single usxe plazstic. Milk BRS11 and Sn1 shows further reduction in weight indicating slower degradation of single-use plastic discs.



Graph representing the evaluation of the efficiency of single use plastic degradation property by selected microbial isolates under laboratory conditions.



Microbial Growth Curve of Milk BRS11, C1 AND Sn1

DISCUSSION

Plastic pollution has become a serious threat to our environment that we are facing today. Whether it's discarded bags, bottles, or wrappers, plastic waste is everywhere—from city streets to the most remote ecosystems. One of the key challenges with plastic is its durability. Most single-use plastics, especially those made from polyethylene, are resistant to degradation and can persist in the environment for hundreds of years. Given the limitations of current waste disposal methods like landfilling, incineration, and recycling, researchers have started to explore biological alternatives to manage plastic waste. Microorganisms—particularly certain bacteria and fungi—have shown promising potential to break down synthetic polymers into simpler, less harmful substances (Shah et al., 2008; Sivan, 2011).

The aim of this study was to examine whether certain bacteria can degrade single-use plastics, and if so, to what extent. We tested three bacterial isolates: one was collected from petroleum-contaminated soil, named Sn1 (from the 10^{-3} dilution of a serial suspension), and the other two—named Milk BRS 11 and C1—were provided by a microbiology laboratory. The rationale behind using an isolate from petroleum-contaminated soil lies in the fact that such environments often host microorganisms that have adapted to break down hydrocarbons. Since plastics like polyethylene are structurally similar to these hydrocarbons, these microbes might also possess the ability to degrade plastic (Yoon et al., 2012; Ojha et al., 2017).

Each of the three isolates was grown on nutrient agar (NA) plates containing small polyethylene plastic discs weighing 5 mg each. Before placing them on the media, the discs were cleaned with ethanol, dried, and sterilized under UV light to eliminate any contaminants. The plates were incubated at room temperature, and the experiment was observed over two time intervals—30 and

45 days. At the end of each incubation period, the plastic discs were carefully removed, washed with distilled water to remove surface biofilm, dried , and reweighed. Any reduction in weight was interpreted as a sign of plastic degradation (Kathiresan, 2003; Restrepo-Flórez et al., 2014).

All three bacterial isolates demonstrated some level of plastic degradation. The most significant results came from the C1 isolate. This strain caused the greatest reduction in the weight of the plastic discs. The environment from which C1 was isolated, or its genetic profile, might have equipped it with efficient enzymes or metabolic pathways to degrade polyethylene more effectively than the other two strains (Restrepo-Flórez et al., 2014).

The Milk BRS 11 isolate also contributed to plastic degradation, albeit to a lesser extent than C1. Its consistent though moderate reduction in plastic weight implies that it may produce enzymes capable of acting on the polymer chains, but perhaps at a slower rate or under more specific conditions. It is possible that this isolate may perform better under optimized settings, or in combination with other bacterial strains that initiate or enhance the degradation process (Shah et al., 2008).

Milk BRS11 showed the least degradation among the three, but its effect was still notable. Its ability to reduce plastic weight, even slightly, suggests that it is capable of interacting with synthetic polymers, likely due to its adaptation to hydrocarbon-rich environments (Ghosh et al., 2013). Although the weight loss was not as significant as with the other two isolates, its contribution supports existing findings that bacteria from polluted environments often have the enzymatic tools to deal with synthetic compounds (Ojha et al., 2017).

These results suggest that certain bacteria do have the ability to degrade plastics, though the efficiency varies. The C1 isolate, in particular, stands out as a promising candidate for further study. It’s possible that it harbors unique enzymes specifically suited to breaking down polyethylene. If so, understanding the enzymatic pathways and conditions that enhance its activity could offer a valuable step forward in the development of bio-based solutions to plastic waste

Further research should focus on identifying the specific enzymes involved in the degradation process through molecular techniques such as gene sequencing and enzyme assays. It would also be beneficial to test the performance of these isolates on other types of plastic or under conditions that mimic natural environments. This would help determine whether they can be used on a larger scale for environmental cleanup or waste treatment facilities. The ultimate goal would be to integrate these findings into sustainable waste management practices that reduce our dependence on conventional disposal methods.

In conclusion, this study adds to the growing body of research suggesting that bacteria can offer a natural, environmentally friendly solution to the plastic waste crisis. While still in its early stages, the ability of bacteria like C1 to significantly degrade polyethylene in controlled conditions gives hope for practical applications in the future. With continued research and optimization, microbial degradation could become a key part of our strategy to combat plastic pollution. The order of bacterial strains degrading single use plastic discs-C1>Sn1>Milk BRS11.

CONCLUSION

Plastic pollution is one of the biggest environmental challenges that are faced by us today, especially because plastics like polyethylene take a very long time to break down naturally (Shah et al., 2008; Sivan, 2011). In this study, it was the aim to see to see if certain bacteria could help tackle this problem by breaking down single-use plastics.

Three different bacterial strains: one from petroleum-contaminated soil(Sn1)and two others from different sources called Milk BRS 11 and C1 was tested. These bacteria were chosen because microbes from polluted environments or diverse habitats often have the tools to degrade tough materials like plastic (Yoon et al., 2012; Ojha et al., 2017).

Over the course of 30 and 45 days, it was watched how well these bacteria could break down small plastic discs made of polyethylene. The results were quite clear. After 30 days, the C1 strain showed significant result , showing a total loss in the weight of the discs. This means C1 has enzymes or abilities that make it really good at digesting plastic in a relatively short time. On the other hand, Milk BRS 11 and the petroleum soil isolate (Sn1) only partially degraded the plastic after 30 days. After 45 days, C1 continued to keep the plastic fully degraded, while the other two strains showed some further, but slower, breakdown.

Interestingly, MILK BRS 11 showed very little to no degradation under these conditions, suggesting it might not be well-suited to breaking down plastic, at least not without some changes in environment or time. The Sn1 strain from contaminated soil did show some ability to degrade plastic, but it was slower compared to C1. So, based on this study, the order of how well these bacteria degrade single-use plastics is: C1 is the most effective, followed by Sn1, with Milk BRS 11 trailing behind-C1>Sn1>Milk BRS11

The strong performance of C1 fits with other studies showing that bacteria adapted to certain environments or with the right enzymes can be very effective at breaking down plastics (Sivan, 2011; Ghosh et al., 2013). The fact that it completely degraded the plastic in just 30 days is very promising and suggests that C1 deserves further research to understand how exactly it works, possibly by identifying the enzymes or genes involved.

To sum up, this study shows that some bacteria really do have the potential to help reduce plastic waste. The C1 strain, in particular, could become a key player in future biotechnological solutions to plastic pollution. While the other strains showed some ability too, their effects were slower or limited. Moving forward, studying these bacteria more deeply and figuring out the best conditions for their activity could open up natural, eco-friendly ways to tackle the growing plastic problem.

REFERENCES

- Ali, A., et al. (2024). Recent Trends in Microbial and Enzymatic Plastic Degradation: A Sustainable Approach. *BMC Sustainable Materials*, 2(11), 1–15.
- Albertsson, A.C. and Karlsson, S. (1990). The influence of biotic and abiotic environments on the degradation of polyethylene. *Progress in Polymer Science*, 15, 177–192.
- Albertsson, A.C., Andersson, A.O. and Karlsson, S. (1987). The mechanism of biodegradation of polyethylene. *Polymer Degradation and Stability*, 18, 73–87.
- Allen, A., Hilliard, N. and Howard, G.T. (1999). Purification and characterization of a soluble polyurethane degrading enzyme. *International Biodeterioration & Biodegradation*, 43, 37–41.
- Dwivedi, A. and Mishra, A. (2022). Plastic degrading microbes: A review on their applications and impact on soil ecosystem. *Environmental Science and Pollution Research.*
- Jayasekara, S. (2012). Screening of Plastic Degrading Bacteria from Dumped Soil Area. *Academia.edu.*
- Kumar, A., et al. (2020). Screening and characterization of plastic degrading bacteria from landfill sites. *Journal of Environmental Management.*
- Ojha, N., et al. (2021). Biodegradation of plastics by fungi and bacteria: A sustainable approach. *Microbial Biotechnology.*
- Purohit, J., et al. (2020). Microplastics in Soil: Impacts and Microbial Diversity and Degradation Mechanisms. *Pedosphere*, 30(4), 1–10.

- Raghul, K., et al. (2016). Isolation and characterization of plastic degrading microbes from municipal landfill. *International Journal of Environmental Science and Technology*.
- Rana, K. and Rana, N. (2020). Isolation and Screening of Plastic Degrading Bacteria from Dumped Soil Area. *International Journal of Current Microbiology and Applied Sciences*, 9(7), 2611–2618.
- Shah, A.A., et al. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology Advances*.
- Shimao, M. (2001). Biodegradation of plastics. *Current Opinion in Biotechnology*.
- Singh, B. and Sharma, N. (2008). Mechanistic implication of plastic degradation. *Journal of Polymers and the Environment*.
- Sivan, A. (2011). New perspectives in plastic biodegradation. *Current Opinion in Biotechnology*.
- Tokiwa, Y., Calabia, B.P., Ugwu, C.U. and Aiba, S. (2009). Biodegradability of plastics. *International Journal of Molecular Sciences*.

PAPER NAME

ISOLATION AND CHARACTERIZATION OF SINGLE USE PLASTIC DEGRADING MICROORGANISMS FROM PETROLEUM CONTAM

WORD COUNT	CHARACTER COUNT
6888 Words	**39183 Characters**
PAGE COUNT	FILE SIZE
24 Pages	**45.7KB**
SUBMISSION DATE	REPORT DATE
May 30, 2025 10:06 AM UTC	**May 30, 2025 10:07 AM UTC**

● 10% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

- 7% Internet database
- 6% Publications database
- Crossref database
- Crossref Posted Content database
- 9% Submitted Works database

● Excluded from Similarity Report

- Bibliographic material
- Quoted material